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Simultaneous determination and validation of doxycycline and neomycin by RP-HPLC in bulk drug and pharmaceutical formulations

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ABSTRACT

An accurate, simple, reproducible and sensitive method for the simultaneous determination of Doxycycline and Neomycin was developed and validated as per ICH Guidelines. Doxycycline and Neomycin were separated by HPLC using a Shimadzu RP-18 column (5µm,250mm x4.6mm i.d) and isocratic elution with a flow rate of 1 mL/min. Mixture of Acetonitrile and Methanol (pH=8.9) (50/50) was used as mobile phase. The detection was at 258 nm wavelength. The retention time of DOXY and NEOM was found to be 5.402 and 2.94 min respectively. The linearity of developed method was achieved in the range of 25-125 µg/mL (r2 = 0.9992) and 5-25 µg/mL (r2 = 0.9995) for Doxycycline and Neomycin respectively. LOD of both the drugs were 0.8406 µg/mL and 0.0427 µg/mL and LOQ was found to be 2.547 µg/mL and 0.129 µg/mL for Doxycycline and Neomycin respectively. Recovery and assay studies of Doxycycline and Neomycin were within 99% to 102% indicating that the proposed method is suitable for routine analysis of ophthalmic formulation.

Keywords: Doxycycline, Neomycin, RP-HPLC, Validation.

INTRODUCTION

Doxycycline is a semisynthetic tetracycline antibiotic derived from oxytetracycline. It may be used orally (dogs, cats, horses) or intravenous (dogs and cats). Doxycycline is Tetracycline antibiotics are broad spectrum and bacteriostatic. Their mechanism of action is through the inhibition of protein synthesis and the alteration of cytoplasmic membrane permeability within the susceptible organism. It is mainly used to treat susceptible bacterial infections and infection caused by a number of other organisms including bartonella, hemoplasma, Chlamydia Elis, Ehrlichia, Anaplasma, and toxoplasma [1].

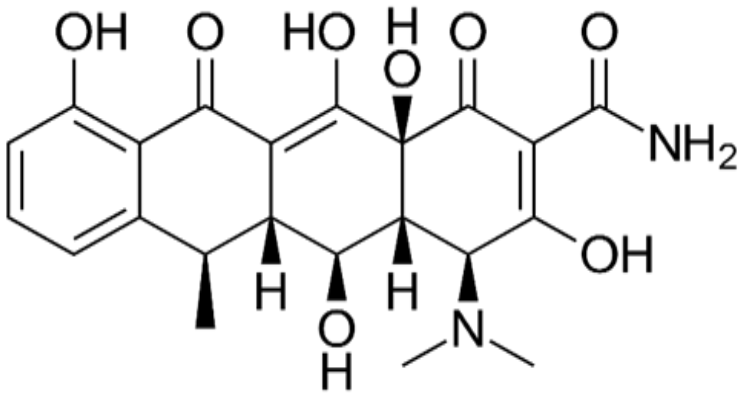

Fig 1: Structure of Doxycycline

Neomycin is bactericidal in action. Similar to other aminoglycosides, it inhibits bacterial protein synthesis through irreversible binding to 30 S ribosomal subunit of susceptible bacteria. Neomycin is actively transported into the bacterial cell where it binds to receptors present on the 30 S ribosomal subunit. This binding interferes with the initiation complex between messenger RNA (mRNA) and the subunit, as a result abnormal and non-functional proteins are formed. Eventually bacteria will die because of the lack of functional proteins [2].

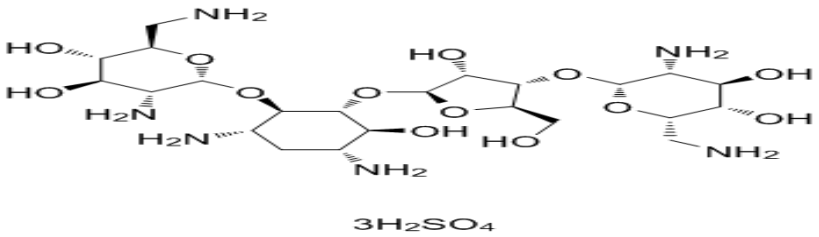

Fig 2: Structure of Neomycin

The combination of Doxycycline and Neomycin is prescribed for certain type of bacterial infection in poultry [3]. On literature survey, it was found that no method has been reported for the simultaneous estimation of Doxycycline and Neomycin in combined dosage forms and no methods is available in the pharmacopoeias. Few analytical methods have been developed for the determination of Doxycycline and Neomycin individually and in combination with other drugs [4-8]. This study makes an attempt to establish simple, sensitive and accurate spectroscopic methods for the simultaneous estimation of Doxycycline and Neomycin in bulk and in combined dosage forms. The present UV-Spectrophotometric method was validated according to (ICH) guidelines [9, 10].

MATERIALS AND METHODS

Instrument

A high-performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software.

Chemicals and regents

Acetonitrile (HPLC grade) and Double distilled water (HPLC grade) procured from Merk ltd, Mumbai, India were used as Mobile phase. All other chemical reagents were of analytical grade.

Drug sample

Standard Doxycycline was obtained as gift samples from Phytopharmaceutical, Plot number 21 ANA 22 industrial Area Jabalpur. Standard

Neomycin was obtained as gift samples from Brawn Laboratories Ltd, Faridabad, and Haryana.

Selection of analytical wavelength

Effect of wavelength on the response factor and on the peak parameters (asymmetry, tailing factor, resolution, theoretical plates) was studied over the wavelength range of 400-200 nm. Satisfactory chromatographic conditions were obtained with a wavelength of 258.0 nm for Multicomponent analysis using HPLC method.

Preparation of mobile phase

A mixture of HPLC grade Acetonitrile and Methanol in the ratio of 50:50 v/v was prepared and pH was adjusted to 8.9 with orthophosphoric acid and filtered through 0.45 µm membrane filter paper and solicited for 20mins.

Preparation of standard stock solution

100 mg each of DOXY and NEOM were weighed separately and transferred into two different 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase by sonication and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/mL of each component (stock A and A' solution).

From the above stock A and A' solution 10mL of aliquot was pipetted out into a 100mL volumetric flask and the volume was made up to the mark with mobile phase to obtain a concentration of 100 µg/mL of Doxycycline (stock B solution) and for Neomycin 10 mL of stock Al was pipetted out into a 100 mL volumetric flask and the volume was made up to the mark with mobile phase to obtain a concentration of 100 µg/mL (stock Bl). From the above stock B and B1 solution further dilution were made to get concentration from 25-125 µg/mL for Doxycycline and 5-25 µg/mL for Neomycin.

Preparation of sample solution

From the formulation, a quantity containing 100mg of Doxycycline was measured accurately and transferred to 100 mL of volumetric flask, volume was made up to mark with solvent to get 1000 µg/mL of Doxycycline (stock A). The contents were solicited for 15 min and the final volume was made up to the mark.

From stock A 10 mL aliquot was taken and dissolved to 100 mL with solvent to get a concentration of 100 µg/mL Doxycycline which also contains 100 µg/mL of Neomycin (Stock B).

Appropriate aliquots were pipetted from the above sample stock "B" solution to get a concentration of 25, 50, 75, 100 and 125 µg/mL of DOXY which also contains 5, 10, 15, 20 and 25 µg/mL of NEOM.

A 20 µl volume of sample was injected in to the sample injector of HPLC system and the chromatogram was recorded under the same chromatographic conditions as described above. The area of each peak was determined at 258 nm and the amount of drug present in the sample was determined.

HPLC CONDITIONS

Table 1: Chromatographic conditions

Instrument	**High Performance Liquid Chromatography SHIMADZU- SPD-20AD Detector**
Injector	Rheodyne 20 µl
Column	Enable C_{18} (250*4.5*5)
Wavelength	UV Detector
Detector	258 nm
Flow rate	1 ml/min
Injection volume	20µl
Mobile phase	Acetonitrile and Methanol (50:50v/v)

METHOD VALIDATION

Validation of the optimized method was performed according to the ICH guidelines.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration

(amount) of analytic in the sample within a given range. The range of analytical procedure is the interval between the upper and lower level of analytic which have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 25-125 µg/mL for DOXY and 5-25 µg/mL for NEOM respectively. Peak areas of DOXY and NEOM were plotted with their respective concentrations and linear regression analysis was performed on the resultant curves. (Fig 6 &7) The regression equation was found to be y = 58802X -49714(r2 = 0.9992) for DOXY and y = 15718X – 22191 (r2 = 0.9995) for NEOM.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations. LOD = 3.3 SD/Slope and LOQ = 10 SD/Slope. Where, SD is the standard deviation of the absorbance of the sample and the slope of the related calibrations curve. The LOD and LOQ of DOXY and NEOM were found to be 0.84067 µg/mL and 0.0427 µg/mL, 2.547 µg/mL and 0.1296 µg/mL respectively.

Accuracy

Recovery studies were carried out by adding 80%, 100% and 120% of the standard drug solution of FLUO and NEOM to the known amount of sample solution by standard addition method. The results obtained for recovery were found to be within the limits. The results are tabulated in Table.2.

Precision

The Intra-day and Inter-day precisions of the proposed method were determined by estimating the corresponding responses three times on the same day and on 3 different days over a period of one week for 3 different concentration and 3 replicates of DOXY and NEOM and reported in terms of relative standard deviation (RSD). Statistical validation of data for Intraday and Inter day precision methods as shown in Table 3.

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing 75µg/ml of Doxycycline and 15µg/ml of Neomycin was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate and wavelengths. The results are shown in Table.4

Ruggedness

The evaluation of ruggedness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 75 µg/ml of Doxycycline and 15µg/ml of Neomycin was injected into sample injector of HPLC two times by different analysts. The results are given in Table.5.

SYSTEM SUITABILITY STUDIES

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), a number of theoretical plates and tailing factor were evaluated for six replicate injections of the drug. The results which are given in Table.7 were within acceptable limits. System suitability test is a pharmacopeia requirement. Summary parameters of the developed methods shown in Table.6.

RESULTS AND DISSCUSSION

The selected drugs Doxycycline and Neomycin in Bulk and Formulation were estimated by RP-HPLC as per ICH guidelines. The methods were validated for all validation parameters as per ICH guidelines. The linearity range in both methods for DOXY and NEOM was 25-125 µg/mL and 5-25 µg/mL respectively. The %RSD for intraday and

inter-day precision was found to be less than 2%. The accuracy of the methods was validated by recovery studies and was found to be significant and within specification limits, with %recovery 98-102%. The assay results were found to be within the acceptable limits.

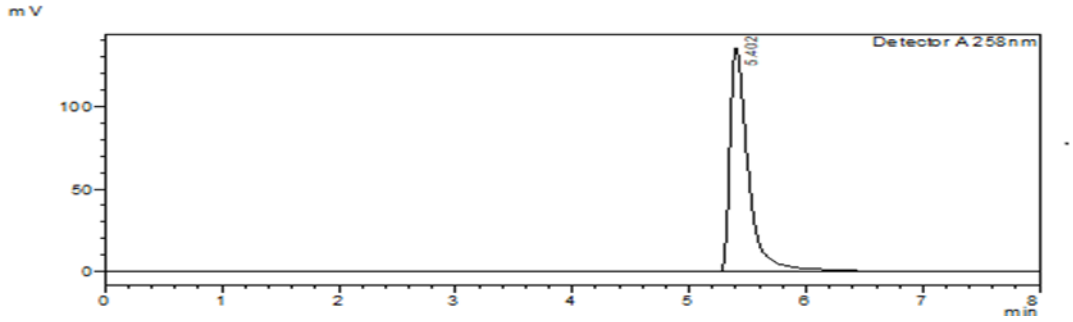

Fig 3: Chromatogram of DOXY.

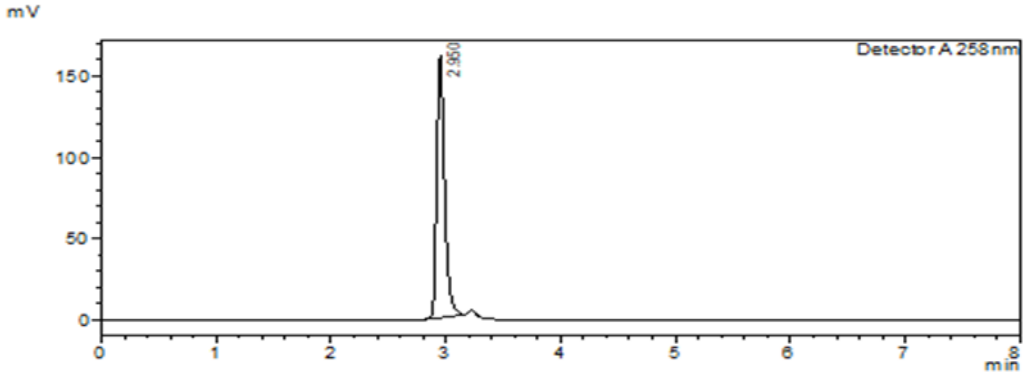

Fig 4: Chromatogram of NEOM.

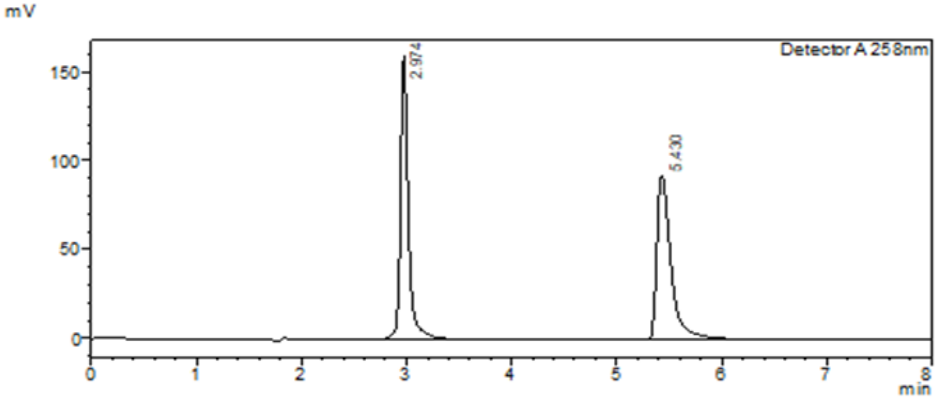

Fig 5: Chromatogram of DOXY and NEOM

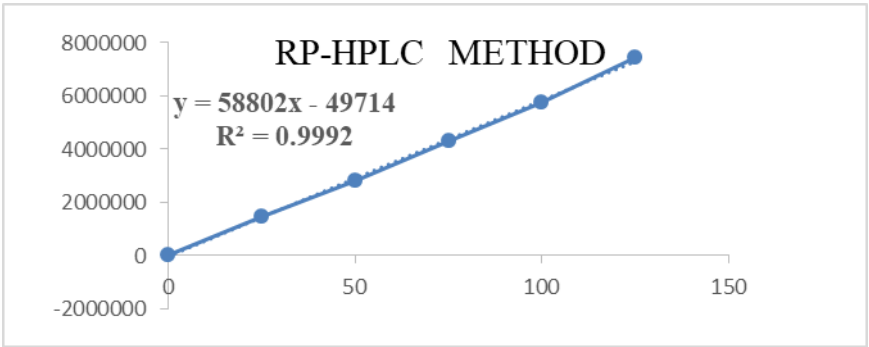

Fig 6: Calibration curve for DOXY at 258 nm by RP-HPLC Method.

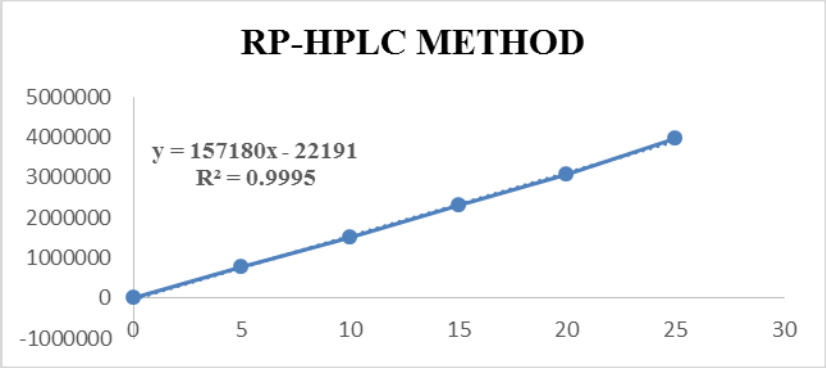

Fig 7: Calibration curve for NEOM at 258 nm by RP-HPLC Method.

Table 2: Statistical Validation Data for Accuracy Determination.

Level-of %recovery	Mean		Std. Deviation		Co-efficient of variation		Standard Error	
	DOXY	NEOM	DOXY	NEOM	DOXY	NEOM	DOXY	NEOM
80%	100.04	100.81	0.0631	0.8630	0.0631	0.8560	0.4888	0.1298
100%	99.93	100.3	0.7427	0.3041	0.7432	0.303	0.5797	0.1664
120%	100.06	100.46	0.1866	1.1942	0.1864	1.1886	0.1543	0.875

Table 3: Statistical Validation Data for Precision.

Precision	Intra-day		Inter-day	
Components	DOXY	NEOM	DOXY	NEOM
Mean	100.24	100.97	100.45	100.37
Sta Deviation	0.197	0.8401	0.374	0.821
RSD	0.1966	0.832	0.3730	0.818
Std Error	0.1045	0.8654	0.488	0.129

Table 4: Data of Robustness.

Method Parameter	Level	Retention time		Tailing Factor	
		DOXY	NEOM	DOXY	NEOM
Flow rate (ml/min)	0.9	5.407	2.98	1.958	1.375
	1	5.402	2.95	1.968	1.393
	1.1	5.392	2.92	1.932	1.373
Wavelength (nm)	256	5.376	2.536	1.943	1.398
	258	5.402	2.95	1.968	1.393
	260	5.458	2.98	1.978	1.341

Table 5: Ruggedness result for variation in Analyst.

Method Parameter	Retention time		Tailing factor	
Analysts	DOXY	NEOM	DOXY	NEOM
Analyst 01	5.409	2.95	1.968	1.393
Analyst 02	5.398	2.89	1.921	1.364

Table 6: Summary of Validation and System suitability parameters of Doxycycline and Neomycin.

Parameters	DOXY	NEOM
Linearity Range µg/mL	25-125	5-25
Slope	58802	15718
Intercept	49714	22191

Regression Coefficient (r^2)	0.9992	0.9995
Limit of Detection (µg/mL)	0.8406	0.0427
Limit of Quantification (µg/mL)	2.547	0.1296
Retention time (min)	5.402	2.92
Tailing factor	1.968	1.393
Resolution factor	12.942	

CONCLUSION

A simple, accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Doxycycline and Neomycin was developed and can be used for routine analysis. Above method was validated as per ICH guidelines.

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REFERENCE

[1]. Barbara Forney, VMD, for veterinary practices, doxycycline for veterinary use.
[2]. https://m.Pdr.net/Mobile/Pages/drug-summary/Neomycin-sulfate-neomycin-sulfate-819.
[3]. https://www.afrimash.com > medicine.
[4]. Monal S S, Uday A D, Rajesh B N. Development and validation of RP-HPLC method for simultaneous estimation of Doxycycline Hyclate and Tinidazole in bulk and tablet dosage form. Int J Pharm Sci, 5(2), 2013, 7666-71.
[5]. Ali M, Shabnam P, Soroush M, Saeid Y. Development and Validation of a stability-indicating RP-HPLC method for Rapid Determination of Doxycycline in Pharmaceutical bulk and dosage forms. Pharm Sci, 22, 2016, 96-104.
[6]. Ghada M H, Mahmoud M E, Randa A A S. Robustness testing in HPLC analysis of Clarithromycin, Norfloxacin, Doxycycline, Tinidazole and Omeprazole in pharmaceutical dosage forms using experimental design. Current Pharm Analysis, 10(1), 2014, 58-70.
[7]. Balaswami B, Peddakotla V R, Bandi S R, Pagidirai S. A New Stability indicating RP-HPlC-PDA method for simultaneous estimation of Neomycin and Flucinolone in pharmaceutical tablet dosage form. Int J Pharm Sci Res, 9(10), 2018, 4187-93.
[8]. Stypulkowska K, Blazewicz A, Fijalek Z, Warowna-Grzeskiewicz M, Srebrzynska K. Determination of Neomycin and Related substance in pharmaceutical preparations by reversed-phase High Performance liquid chromatography with mass spectrometry and charged aerosol detection. J Pharm and Biomed Anal, 76, 2013, 207-14.
[9]. ICH, Q2A, Test on validation of Analytical Procedures, International Conference on Harmonization, Geneva, 1994, 1-5.
[10]. ICH, Q2B, Validation of Analytical procedure: Methodology, International Conference on Harmonization, Geneva, 1996, 1-8.